



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 447-451
Received: 25-09-2019
Accepted: 27-10-2019

Hassanain Hataf Jaber
Department of Biotechnology,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur,
Andhra Pradesh, India

K Rajyalakshmi
Department of Biotechnology,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur,
Andhra Pradesh, India

A Krishna Satya
Department of Biotechnology,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur,
Andhra Pradesh, India

Corresponding Author:
A Krishna Satya
Department of Biotechnology,
Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur,
Andhra Pradesh, India

Screening of a nearly threatened woody tree species *Pterospermum xylocarpum* for antioxidant and anti-inflammatory activities

Hassanain Hataf Jaber, K Rajyalakshmi and A Krishna Satya

Abstract

Pterospermum xylocarpum is a woody tree species belonging to the family *Sterculiaceae*. This plant is commonly known as "Tada Chettu" (in Telugu). It is widely distributed in India, south East Asia, Pakistan and North America. *P. xylocarpum* is common in deciduous forests and it is of 5-8mts tall tree. It is merely distributed in the tropical regions, found in the hills of Andhra Pradesh & Tamil Nadu. There is no recorded evidence on its phytochemicals or its medicinal compounds; hence we preferred this massive tree species for its phytochemical & bioactive compound study. The plant was selected for the screening of crude extracts [Hexane, Chloroform, Methanol and Aqueous (Water) extracts] for their antioxidant and anti-inflammatory activities. The antioxidant activity of the extracts was determined using free radical scavenging method by using DPPH (1,1-diphenyl-2-picrylhydrazyl) as well as by estimating the ion reducing power through FRAP assay (Ferric reducing antioxidant power). Anti-inflammatory activity of extracts was done by using 5-Lipoxygenase inhibitory assay. Plant has shown good antioxidant activity in the methanol extract. Antioxidant activity of four extracts was determined by the two methods and IC₅₀ was also calculated for those extracts. In the present study we have calculated the *in-vitro* lipoxygenase activity for the four extracts with the five concentrations. The activity of the methanol extract was good and showed equal results with standard Indomethacin.

Keywords: *Pterospermum xylocarpum*, antioxidant activity, DPPH, anti-inflammatory activity

Introduction

The present study is focused on Threatened plant of Andhra Pradesh *Pterospermum xylocarpum* noted by Surya Narayana, B and Srinivasa Rao, A in Threatened Medicinal plants of Andhra Pradesh 2002^[1] and noted as Nearly Threatened by Sudhakar reddy in Catalogue of invasive alien flora of India. Life Science Journal, Vol 5, No 2, 2008^[2]. *Pterospermum xylocarpum* is a woody tree species belonging to the family *Sterculiaceae*. This plant is commonly known as "Tada Chettu" (in Telugu). It is widely distributed in India, south East Asia, Pakistan and North America. *P. xylocarpum* is common in deciduous forests and it is of 5-8mts tall tree. It is merely distributed in the tropical regions, found in the hills of Andhra Pradesh & Tamilnadu. There is no recorded evidence on its phytochemicals or its medicinal compounds; hence we preferred this massive tree species for its phytochemical & bioactive compound study. After detailed research survey and review of literature it is observed that the plant *Pterospermum xylocarpum* even though distributed widely and locally recognized for ethno botanical and pharmacological applications, the plant was less explored scientifically. It is evident from the past research reports that the plant is the richest source of alkaloids^[3]. As ~60% of known phytochemicals belongs to the class of alkaloids and are well known for therapeutic applications, the plant *Pterospermum xylocarpum* was selected for the screening of crude extracts [Hexane, Chloroform, Methanol, and Aqueous (Water) extracts] for their antioxidant and anti-inflammatory activities. Screening with crude extracts is followed by isolation and identification of primary factors/ compounds responsible for particular pharmacological activity.

Materials and Methods

Collection of plant material

The medicinal plant *Pterospermum xylocarpum* leaves were collected from the Seshachalam forest area. The collected plant material was shade dried for two weeks, powdered, labelled and stored in a dry, cool place for further studies.

Chemicals and Reagents

All the chemicals, solvents and reagents used were of analytical grade and procured from Merck, Sigma, Sd-fine and SRL.

Soxhlet extraction

The dried plant material was packed into soxhlet apparatus, subjected to successive extraction with solvents such as Hexane, Chloroform, Methanol and Aqueous (Water). For this purpose, the plant material of 300gms was packed into the extractor and fitted with apparatus. The respective solvent was filled and apparatus was operated at 45°C until 35 cycles were run. The experiment was repeated several times with fresh dry powder until ample amount of crude extract was collected. After collection of extract, the remaining plant powder was removed, dried and once again loaded with another successive solvent *i.e.*, from non-polar to polar. The isolated fraction of each extract was labelled with their respective solvent names and subjected to further studies.

Quantitative% Yield of *Pterospermum xylocarpum*

To evaluate the quantitative % yield, the extracts after rotary evaporation were taken into Petri plates and weighed^[4]. The yield of extract was calculated as per the formula which is given below.

$$\%yield = \frac{\text{Weight of the extract (gms)} \times 100}{\text{Weight of the sample used for extraction (gms)}}$$

Phytochemical screening

To carry out the different phytochemical screenings required reagents such as Benedict's reagent, Dragendroff's reagent, Fehling's solution A & B, Liebermann-Burchard reagent, Mayer's reagent and Molisch reagent were prepared as per standard procedures and protocols^[5].

Test for flavonoids

Flavonoids are the polyphenolic compounds with 15 carbon atoms, water soluble and commonly present in plants. The presence of flavonoids was tested using different tests such as a) Ferric chloride test, b) Shinoda's test c) Sodium hydroxide test and d) Lead acetate test.

a) Ferric chloride test

2mL of test solution was boiled with distilled water and filtered followed by addition of few drops of 10% FeCl₃.6H₂O solution. A greenish-blue or violet colouration indicates the presence of a phenolic hydroxyl group^[6].

b) Shinoda's test

5gms of each extract was dissolved in ethanol, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of Conc. HCl. The pink, orange, or red to purple colouration indicates the presence of flavonoids^[6].

c) Sodium hydroxide test

0.2gms of the extract was dissolved in distilled water and filtered. To this, 2mL of 10% aqueous NaOH solution was added to produce yellow colouration. A change in colour from yellow to colourless on the addition of dilute HCl was the indication for the presence of flavonoids^[6].

d) Lead acetate test

0.5gms of the extract was dissolved in distilled water and filtered. To the filtrate 3mL of Pb (CH₃COO)₂ [Lead acetate] solution was added and mixed well. The appearance of a buff-coloured precipitate indicates the presence of flavonoids^[6].

Test for alkaloids

Alkaloids are the class of phytochemicals with a nitrogenous organic skeleton and widely distributed with diverse medicinal functions. The presence of alkaloids was tested with a) Dragendroff's reagent test and b) Mayer's reagent test.

a) Dragendroff's reagent test

Dragendroff Reagent: 1.7gms of Bi (NO₃)₃.5H₂O [Bismuth nitrate] and 20gms of C₄H₆O₆ [tartaric acid] was dissolved in 80mL of water and mixed with a solution containing 16gms of KI [potassium iodide] dissolved in 40mL of water.

5gms of crude extract was stirred with 1% aqueous HCl in a water bath at 60°C and then filtered. To 1mL of the filtrate, few drops of Dragendroff's reagent were added. Orange-Red precipitate was taken as positive^[6].

b) Mayer's reagent test

Mayers Reagent: To 60mL of water 1.36gms of Mercuric iodide was added, mixed well and added with a solution 5gms Potassium iodide dissolved in 20mL of water.

To 1mL of the filtrate, few drops of Mayer's reagent were added and appearance of buff-coloured precipitate will be taken as the positive test for alkaloids^[7].

Test for soluble starch

0.2gms of the extract was boiled in 1mL of 5% KOH, cooled and acidified with H₂SO₄. Yellow colouration indicates the presence of soluble starch^[8].

Test for Saponins

Saponins are the amphipathic glycosides with foaming characteristics. The presence of Saponins was tested by using Frothing test.

Frothing test: 0.5gms of the extract was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins^[9].

Test for terpenoids

Terpenoids or isoprenoids are the largest class of small organic molecules present plants with diverse functions. Terpenoids are derived from terpenes after structural modification.

5gms of crude extract was dissolved in ethanol. To this, 1mL of acetic acid was added followed by Conc. H₂SO₄. A change in colour from pink to greenish confirms the presence of terpenoids^[9].

Test for steroids

The steroids are the organic compounds with four fused ring structure found in plants. The presence of steroids was tested with four tests such as a) Salkowski test, b) Keller-Killiani test and c) Liebermann-Burchard test.

a) Salkowski test

0.2gms of the extract was dissolved in 2mL of chloroform and to this Conc. H₂SO₄ was added. The development of reddish brown colour at interphase indicates the presence of steroids^[10].

b) Keller-Killiani test

0.5mL of test solution was mixed with 2mL of 3.5% FeCl₃, 2mL of Conc. H₂SO₄ and a small amount of glacial acetic acid carefully. The appearance of a reddish brown ring at interphase is a positive indication for the presence of steroids.

c) Liebermann-Burchard test

0.2gms of the extract was mixed with 2mL of acetic acid, cooled well in ice followed by the addition of Conc.H₂SO₄ carefully. Color development from violet to blue or bluish-green indicates the presence of a steroidal ring (*i.e.*, aglycone portion of cardiac glycoside).

Test for carbohydrates**a) Molisch's test**

The extract was dissolved in distilled water and added with 2mL of Molisch's reagent and 1mL of Conc. H₂SO₄ was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5mL of distilled water. Formation of a dull violet colour at the interphase of the two layers indicates the positive test for carbohydrates.

b) Fehling's test (for free reducing sugars)

The crude extracts were treated with 5mL of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of free reducing sugars.

c) Fehling's test (for combined reducing sugars)

0.5gms of the extract was hydrolyzed by boiling with 5mL of dilute Hydro chloric acid and the resulting solution neutralized with Sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on a water bath for 2 minutes at 60°C. The appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars.

d) Barfoed's test (for monosaccharides)

0.5gms of the extract was dissolved in distilled water and filtered. To 1mL of the filtrate, 1mL of Barfoed's reagent was added and then heated in a water bath at 60°C for 2 minutes. A reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharides.

Detection of anthroquinones**a) Free anthroquinones test: (Borntrager's test)**

The extract of the plant material (equivalent to 100mg) was shaken vigorously with 10mL of benzene, filtered and 5mL of 10% ammonia solution added to the filtrate. Presence of a pink, red, or violet colour in the ammonia (lower) phase indicated the presence of free anthraquinones.

b) Modified Borntrager's test

The extract was treated with Ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of tannins

0.5gms of the extract was dissolved in 10mL of distilled water and stirred for few minutes and filtered. To 2mL of the filtrate 1-2 drops of 1% Ferric chloride solution was added.

Appearance of blue colour indicates the gallic tannins and greenish black colour appearance indicates the presence of catecholic tannins^[11].

Antioxidant activity

The antioxidant activity of the extracts was determined using free radical scavenging method by using DPPH (1,1-diphenyl-2-picrylhydrazyl) as well as by estimating the ion reducing power through FRAP assay (Ferric reducing antioxidant power).

DPPH method

For estimating the antioxidant activity using DPPH, different concentrations of the extract such as 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL and 500µg/mL were dissolved in DMSO followed by addition of 4mL of the 0.004% (w/v) DPPH dissolved in Methanol. The reaction mixture was kept for incubation in dark for 30 minutes. Ascorbic acid was used as a standard. The absorbance was measured at 517nm using Thermo scientific UV-Visible spectrophotometer^[12].

The DPPH scavenging activity (%) was calculated as per the formula (Yen GC *et al.*, 1994).

$$\text{Inhibition \%} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of the DPPH radical with Methanol, A_{sample} is the absorbance of DPPH radical with sample extract/standard.

Ion reducing power assay (FRAP method)

The Frap method was performed by using a modified method developed by^[13, 14]. The following stock reagents were prepared

(A) 300 mM acetate buffer: 3.1gms of sodium acetate trihydrate was dissolved in 16mL of glacial acetic acid. The final volume was made up of 1000mL with distilled water.

(B) 10mM TPTZ solution: 0.3123gms of 2, 4, 6-tripyridyl-s-triazine (TPTZ) was dissolved in 100mL of 40mM HCl.

(C) 20mM ferric chloride solution: 5.406gms of FeCl₃.6H₂O was dissolved in 1000mL of distilled water.

A working reagent was made by mixing stock reagents A, B, and C in a 10:1:1 ratio. The solution was then warmed at 37°C for 10 minutes.

100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL and 500µg/mL concentrated samples were prepared in their respective solvents. From this, 200µL sample was mixed with 3.8mL of working reagent and incubated in the dark at room temperature for 30 minutes. After incubation the optical density was measured at 593nm, reagent alone was used as a blank. All the experiments were conducted in triplicates and mean values were taken.

Anti-inflammatory Activity**Evaluation of 5-Lipoxygenase Inhibitory Activity**

Anti-inflammatory activity of extracts was done by using 5-Lipoxygenase inhibitory assay.

Chemicals and reagents

Potato 5-LOX was purchased from Biosense Ltd., Norway. Linoleic acid and other chemicals were procured from Merck Specialities Pvt. Ltd.

5-Lox enzyme inhibitory activity of selected plant extracts was measured by a method developed by Reddanna [15] with some modifications done.

The assay mixture contained 80 mM linoleic acid and 10 µl 5-lipoxygenase enzyme in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mixture to substrate (linoleic acid) and the enzyme activity was monitored as the increase in absorbance at 234 nm. The reaction was monitored for 2 min and the inhibition studies of the extracts were measured by incubating various concentrations of test extracts with enzyme buffer mixture for 2 min before addition of the substrate. The assay was performed in triplicate and mean values were used for the calculation. Percentage inhibition was calculated by comparing slope or increase in absorbance of the test substance with that of control enzyme activity.

The percentage inhibition was calculated by following equation

$$\% \text{ of inhibition} = [(A_0 - A_s) / A_0] \times 100$$

Where, A_0 is the absorbance of the control and A_s is the absorbance of the plant sample

Results and Discussion

Antioxidant Activity

DPPH radical scavenging antioxidant activity of *Pterospermum xylocarpum*

The four extracts Hexane, Acetone, Methanol and Aqueous with five concentrations were studied for two antioxidant studies and the experiment was performed in triplicate and values were entered in to the records.

The scavenging activity of DPPH of four extracts was moderate and they were dose dependent. Hexane extract has shown the low antioxidant activity and the IC_{50} 438.25 µ g. The acetone extract is having better activity than the hexane extract and it was 386.95 µ g. The Methanol extract was the most efficient, having the lowest IC_{50} value 265.9 µ g. Methanol and Aqueous extracts were having same IC_{50} values, but due to the inorganic nature of aqueous extract we have considered the methanol extract for the further studies.

The total phenol content and the flavonoid contents of the four extracts were analysed in the earlier studies and it was stated that Methanol extract was having the highest phenol and flavonoid content by its nature of polarity. There is evidence that as the polarity of the solvent increases and dissolution of the phytochemical constituents also increases.

The DPPH radical scavenging activity of four extracts and five concentrations were studied and the activity was dose dependent, when the concentration increases the activity also

increased. The order has slightly changed for the FRAP assay and it also been investigated for the better interpretation of antioxidant activity and to calculate the results based on their nature of the extracts.

FRAP radical scavenging antioxidant activity of *Pterospermum xylocarpum*

In this study also the plant extracts Hexane, Acetone, Methanol and Aqueous with five concentrations 100 to 500 µg/ml were taken to evaluate antioxidant activity the four extracts have showed dose dependent activity.

The hexane extract has the highest IC_{50} which was 397.43 µg when compared with the DPPH the activity was much similar with the activity. Acetone extract was 338.18 µg of its IC_{50} value. The methanol extract was the most efficient that it showed the lowest IC_{50} value and has highest anti-oxidant activity. The IC_{50} value of Methanol extract is 129.69 µg and it is very close to the standard ascorbic acid.

Anti-inflammatory Activity of *Pterospermum xylocarpum*

Inflammation is calculated by *in-vitro* lipoxygenase assay. The inflammatory disorders of commonly prescribed agents are NSAIDs like fever, arthritis, pain, gout etc. The hexane extract was having low IC_{50} value which was 421 µ g/ml, acetone extract IC_{50} value was 342.55 µ g/ml and methanol extract has the lowest IC_{50} value that is almost near to the standard drug. The IC_{50} value of methanol extract was 148.09 µ g/ml and it was almost nearer to the standard drug. The IC_{50} value for the aqueous extract was 206.8 µ g/ml.

In comparison of four extracts and its anti-inflammatory activity with the standard drug Methanol extract has shown good anti-inflammation activity and it has the ability to influence the lipoxygenase to resist the inflammation.

Conclusion

In the present study *Pterospermum xylocarpum* is an efficient medicinal plant that has shown good antioxidant activity in the methanol extract. The antioxidant activity was performed by two methods DPPH radical scavenging activity and FRAP assay. Antioxidant activity of four extracts was determined by the two methods and IC_{50} was also calculated for those extracts.

The lipoxygenase is the enzyme that mediates the inflammatory response, by inhibiting the 5-lipoxygenase enzyme will stop the inflammatory response in the human body. In the present study we have calculated the *in-vitro* lipoxygenase activity for the four extracts with the five concentrations. The activity of the methanol extract was good and equal to the standard Indomethacin.

Table 1: DPPH radical scavenging antioxidant activity of *Pterospermum xylocarpum*

Conc (µg/ml)	Hexane extract	IC_{50}	Acetone extract	IC_{50}	Methanol extract	IC_{50}	Aqueous extract	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
100	29.2	438.25	30.5	386.95	42.8	265.9	35.5	269.8	50.67	106.62
200	32.4		36.7		48.3		41.2		53.25	
300	40.8		45.5		62.3		47.6		79.08	
400	48.4		51.6		78.8		52.3		83.51	
500	53.8		57.7		85.8		59.7		91.26	

Table 2: FRAP radical scavenging antioxidant activity of *Pterospermum xylocarpum*

Conc µg/ml	Hexane extract	IC_{50}	Acetone extract	IC_{50}	Methanol extract	IC_{50}	Aqueous extract	IC_{50}	Ascorbic Acid	IC_{50}
100	21.45	397.43	21.62	338.18	45.17	129.69	29.4	233.10	50.67	106.62
200	31.33		34.94		52.44		47.38		53.25	
300	43.3		44.81		68.47		62.59		79.08	

400	51.12		58.98		82.07		62.03		83.51
500	58.66		66.92		87.69		71.97		91.26

Table 3: Anti-inflammatory activity of *Pterospermum xylocarpum*

S. No	Conc	Hexaneextract	IC50	Acetoneextract	IC50	Methanolextract	IC50	Aqueousextract	IC50	Indomethacin	IC50
1	100	21.76	421 µg/ml	30.76	342.55 µg/ml	41.57	148.09 µg/ml	35.71	206.8 µg/ml	44.25	138.84 µg/ml
2	200	28.78		38.78		49.33		48.78			
3	300	39.91		58.77		79.88		67.82			
4	400	46.3		62.71		82.4		74.74			
5	500	52.61		64.71		83.85		76.81			

References

- Surya Narayana B, Srinivasa Rao A. Flora of Nellore District. Andhra Pradesh. (Publication Maharashtra), 2002.
- Sudhakar Reddy. Catalogue of invasive alien flora of India. Life Science Journal. 2008; 5:2.
- Narendra K, Sowjanya KM, Swathi J, Krishna Satya A A. Phytochemical Evaluation & Antimicrobial Efficiency Of Threatened Medicinal Plant Of Andhra Pradesh *Pterospermum xylocarpum* (Thada Tree) International Research Journal of Pharmacy, 2013, 4(2).
- Okigbo RN, Ramesh Putheti, Achusi CT. Post-Harvest Deterioration of Cassava and its Control Using Extracts of *Azadirachta indica* and *Aframomum melegueta*, E- Journal of Chemistry. 2009; 6(4):1274-1280.
- Harborne JB, Methods of Plant Analysis, Phytochemical Methods, pp. 1-36.
- Trease GE, Evans WC. Pharmacognosy. 11th Edition, Bailliere Tindall, London, 1989, 45-50.
- Brain KR, Jones BE, Turner TD. Application of densitometry to the qualitative and quantitative evaluation of pharmaceutical colourants- Journal of Chromatography A, 1975, Elsevier.
- Vishnoi NR. Advanced Practical Chemistry. Yikas Publication House, PVT Ltd., Ghaziabad-India, 1979, 447-449.
- Sofowora A. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd; Screening Plants for Bioactive Agents, 1993, pp. 134-156.
- Dr. Rimjhim Sheel, Kumari Nisha, Prof. Jainendra Kumar Preliminary Phytochemical Screening of Methanolic Extract of *Clerodendrum infortunatum* January IOSR Journal of Applied Chemistry. 2014; 7(1):10-13.
- Ramesa ShafiBhat, Sooad Al-daihan. Antimicrobial activity of *Litchi chinensis* and *Nephelium lappaceum* aqueous seed extracts against some pathogenic bacterial strains Journal of King Saud University – Science Volume. 2014; 26(1):79-82.
- Harsahay Meena, Hemant Kumar Pandey, Pankaj Pandey, Mahesh Chand Arya, Zakwan Ahmed. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Bacopa monnieri* and *Centella asiatica*. Indian Journal Pharmacology. 2012; 44(1):114-117.
- Kriengsak Thaiponga, Unaroj Boonprakob, Kevin Crosby, Luis Cisneros-Zevallos, David Hawkins Byrne. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 2006; 19(6-7):669-675.
- Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry Volume. 2006; 94(4):550-557.
- Reddanna P, Whelan J, Maddipati K, Reddy CC. Purification of arachidonate 5-lipoxygenase from potato tubers. Methods Enzymol. 1990; 187:268-277.